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13. ABSTRACT (Maximum 200 Words) This project will assess the association between breast cancer risk and polymorphisms of the androgen receptor (AR) and vitamin D receptor (VDR) genes among subjects in a population-based case-control study in Sweden. From this study population, breast cancer cases and control women have been randomly selected for genomic DNA analysis. The collection of blood or tissue specimens has been funded from other sources; this award is for the measurement of the AR and VDR polymorphisms on 300 cases and 300 controls who never used HRT, and a similar number of cases and controls who used HRT for 4 years of more. Information on these polymorphisms will be incorporated into the established subject database, and odds ratios summarizing the associations with breast cancer risk will be computed. In the previous years of the project, the work was organized, a tracking database for subject recruitment and specimen accrual was built, and recruitment into the molecular epidemiology study completed. The laboratory investigators have completed DNA extraction and amplification from virtually all available specimens, and have as well completed most of the assays. All laboratory analyses will be completed in December 2001. Under a no-cost extension, data analysis and reporting will be completed in 2002.				
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Introduction

This project aims to assess the association between the risk of breast cancer and germline polymorphisms of the androgen (AR) and vitamin D receptor (VDR) genes among subjects in a recently completed population-based case-control study in Sweden. A total of 3879 cases and 3527 controls took part in the questionnaire phase of the study, providing data regarding use of exogenous hormones and other life style factors. From this study population, breast cancer cases and control women have been randomly selected for genomic DNA analysis. The collection of blood or tissue specimens for DNA has been funded from other sources; this award is for the measurement of the AR and VDR on 300 cases and 300 controls who never used HRT, and 300 cases and 300 controls who used HRT for 4 years or more. Information on these polymorphisms will be incorporated into the established subject database, and odds ratios summarizing the associations with breast cancer risk will be computed.

Body of Progress Report

Under previous funding from other sources, all questionnaire data have been obtained and organized, and several manuscripts dealing with the questionnaire data have been published (see previous reports).

Administrative and Informatics Preliminaries:

The grantee organization, Dartmouth College, is maintaining a subcontract with the Karolinska Institutet for the collaborative work described in our proposal. The Karolinska Institutet has an on-going relationship with investigators at Uppsala University in Uppsala, Sweden, where the molecular analysis are being conducted. A tracking database for subject recruitment and specimen accrual was built for the study.

Recontacting study subjects to obtain germline DNA for analyses:

This work, funded by other awards from the National Institutes of Health and from the Army Medical Research and Materiel Command Breast Cancer Research Program, has been completed. We attempted to obtain blood or tissue sample from 1801 breast cancer patients, selected from the 3879 cases initially enrolled in the study. We have been successful in obtaining a source of DNA for 87.1% (1569) of the selected cases. 1322 (73.4% of those sampled) donated a blood sample, and we were able to access a tissue sample from an additional 247 cases (13.7%) who had died or declined to donate a blood sample. Eighty patients (4.4%) refused to participate in this phase of the study, and we were unable to obtain samples from 152 others due to other reasons (see table 1 below).

We attempted to obtain blood samples from 1712 control women (without previous breast cancer), selected from the 3527 control women initially enrolled in the study. (Since these subjects had not had breast cancer, there was no ready tissue source of DNA for them.) We have obtained blood samples from 1272 control women (74.3%). 349 control subjects (20.4%) declined to participate, and 91 (5.3%) had died before we could contact them.

Table 1. Participation frequencies among breast cancer cases and controls.

	Number selected	Donated Blood (%)	Allowed use of Tissue %	Refused (%)	No specimen for other reason ¹ (%)	Total participation, %
Cases	1801	1322 (73.4)	247 (13.7)	80 (4.4)	152 (8.4)	87.1
Controls	1712	1272 (74.3)	--	349 (20.4)	91 (5.3)	74.2

¹ Other reasons for non-participation (cases): Declined blood draw and died before contact regarding use of tissue, blood sample lost, tissue sample could not be obtained, and subject could not be traced. Other reasons for non-participation (controls): Subject died before contact.

Laboratory analysis

This Army award will support the analysis of a subgroup of these subjects (600 cases and 600 controls); analysis for the others will be supported by other sources (NIH and another USAMRMC award).

After being entered into the administrative tracking system, all blood/tissue specimens have been sent to the laboratory in Uppsala, Sweden. DNA has been extracted from virtually all the blood samples, and from the overwhelming majority of tissue samples. Currently, the laboratory is completing final assays and checking results. The table below details our success in extracting and amplifying the DNA from the specimens. For a few tissue samples the quality of the DNA extracted has not supported successful amplification; continued attempts are being made to achieve success.

Table 2. Frequency to date of successful sample genotyping

	Blood samples	Tissue samples
Androgen receptor	99.5%	85%
Vitamin D receptor	99.2%	59%

All laboratory efforts will be completed in December, 2001, and the data conveyed to Stockholm for incorporation into the study database.

Analysis and Reporting

Statistical analysis and writing of scientific manuscripts and reports will be accomplished as soon as the laboratory data set is complete, now projected for January, 2002. We anticipate completing the first reports by June, 2002, and all relevant reports by December 31, 2002.

Training

The project has played a role in the training of two investigators. The data generated will be part of the Ph.D. thesis of Ms. Sara Wedren, and the work is also part of the activities for the post-doctoral fellowship of Dr. Elisabete Weiderpass.

Key Research Accomplishments

- completion of organizational prerequisites
- construction of a detailed administrative database
- successful completion of recruitment of subjects to the molecular epidemiology study
- extraction and amplification of DNA from the specimens
- completion of laboratory analyses for the majority of subjects

Reportable Outcomes

a) Manuscripts, abstracts, presentations:

The post-doctoral student working in the project (Elisabete Weiderpass, Karolinska Institutet, Stockholm) presented two posters describing the project at the 'Era of Hope' conference (Atlanta, Georgia, June, 2000).

b) Patents and licenses applied for and/or issue

None

c) Degrees obtained that are supported by this award:

The Ph.D. thesis work of a graduate student in Cancer Epidemiology, Ms. Sara Wedren (Karolinska Institutet, Stockholm, Sweden), is partially drawn from this project. She is expected to defend her thesis, thus obtaining her Ph.D. degree, during the year 2002.

d) Development of cell lines, tissue or serum repositories:

A biological bank containing DNA samples from breast cancer patients and control women is being created and will be maintained using financial resources obtained elsewhere. The creation and maintenance of the biological bank follows the Swedish law for storage of biological samples for scientific proposals.

e) Informatics such as databases and animal models, etc:

- An administrative database containing information about study subjects has been created at the Karolinska Institutet;
- A database containing questionnaire information from breast cancer case patients and control women has been created at the Karolinska Institutet, and the questionnaire information has been already checked out and corrected for typing errors and logical inconsistencies.
- Programs (using SAS programs) allowing calculation of time of use of different sorts of postmenopausal hormones have been developed and tested at the Karolinska Institutet.
- Databases containing results from the laboratory analyses have been created in Uppsala, Sweden, where the laboratory analysis is being performed. These files will be transferred to the Karolinska Institutet at the completion of the laboratory analysis (expected date: end of December, 2001).

f) Funding applied for based on work supported by this award:

A post-doctoral fellowship in Cancer Epidemiology was granted from the Swedish Cancer Society to Dr. Elisabete Weiderpass (Karolinska Institutet, Stockholm, Sweden) to work in this project. This fellowship partially supported her salary from January to December 2000.

- g) Employment or research opportunities applied for and/or received based on experience/training supported by this award:

The graduate student working on the project, Ms. Sara Wedren, obtained a training fellowship from the Karolinska Hospital, to complete her medical training. She qualified for this fellowship program in part because of her experience in this project.

The post-doctoral fellow working on the project, Dr. Elisabete Weiderpass, has being appointed Docent in Cancer Epidemiology at the Karolinska Institutet, Sweden, in September 2000. She qualified for this position in part because of the experience she acquired working in the project.

Conclusions

Although the work to date has been substantial, laboratory delays have necessitated postponement in the completion of work for this project. A no-cost one-year extension has been obtained. We anticipate completion of reporting during calendar year 2002.